Bioactive compounds and growth performance of endemic medicinal plants (Cuphea spp.) cultured in aquaponic system

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Abstract

Aquaculture wastewaters are associated with modifying the phytochemical profile in plants when watered with them, thus, aquaponics is a way to improve medicinal plants' quality. This study aimed to analyse the effect caused by a small-scale aquaponic system integrated with Koi carp in the growth performance and modification of bioactive compounds in *Cuphea hyssopifolia* and *Cuphea cyanea*. The results showed the aquaponic system design is suitable for keeping *Cuphea* spp. in a greenhouse or indoor conditions. No statistically significant differences were found in the growth performance in both *Cuphea* spp. The results for *C. hyssopifolia* in aquaponic showed that approximately 76% of phenols and more than half of the flavonoids remained in the dry basis of the plant cultivated in aquaponics compared to conventional culture. The apigenin content increased by > 60% (1.63 mg g^{-1}). The results for *C. cyanea* in aquaponic showed that the phenolic content remained above, and the flavonoids decreased by 53%. The apigenin content decreased by 40% (0.89 mg g^{-1}). The outcomes indicate that aquaponics can promote biostimulation of medicinal plants and increase their bioactive compounds, however, this effect does not occur in the same way between species.

Introduction

The nutrient content of aquaculture wastewater is composed of metabolic waste from fish by respiration, urine, feces, and unconsumed dissolved food (Oladimeji et al., 2020). This effluent also has a load of microorganisms and, together with the dissolved organic molecules, can help activate secondary metabolism, defences, and plant growth when used as irrigation, and consequently, increase the quality of vegetables by modifying their phytochemical profile [1,2]. According to Braglia et al. (2022), the antioxidant activity of aquaponic herbal crops (i.e., *Ocimum basilicum* and *Petroselinum crispum*) was significantly higher than crops grown organically in soil. Suhl et al. (2016) and Delaide et al. (2019) found the same effect in lowering Blossom-End Rot symptoms; this was related to the microorganism and the dissolved organic molecules in wastewater acting as biostimulants in the crops. Therefore, aquaponics cultivation presents an alternative as biostimulation for enhancing the nutritional value and stress tolerance of crops.

Aquaponics are part of a broader area, the Integrated Agri-aquaculture Systems (IAAS) join in two of the most productive sectors in their area: aquaculture and hydroponics; wastewater from aquaculture ponds is directed toward crops and provides them with at least 50% of the nutrients required for their growth [5]. In this way, aquaponics solves some problems of conventional agricultural systems, such as low productivity, limited product diversification, and waste of nutrients and water [6]. Moreover, aquaponic systems are an alternative for sustainable and organic production because they impact the environment to a lesser extent compared to aquaculture and traditional hydroponics [7–11]. According to Yang and Kim (2020), vegetables produced in aquaponic systems have higher fruiting than those grown in hydroponics. Nevertheless, until now, the biostimulation effect of aquaponics has not been tested in medicinal plants.

Cuphea is a plant genus with approximately 260 species that has a significant role in Mexican ethnopharmacology (Graham & Sosa, 1991; Santos et al., 2021). The species in this genus are known due to their content of medium-chain fatty acids in the seeds, such as capric, lauric, and myristic, a profile comparable to that of *Cocos nucifera*[15–17]. However, its most significant potential is in its content of phytochemicals associated with antimicrobial, antiviral, and cytotoxic activities [18]. Other popular uses range from treating dermatological conditions, like skin tumours, pain, inflammation, and wounds, to diarrhea and stomach infections [19]. One promising species is *Cuphea hyssopifolia*, also called “Falso brezo (false heather)”, which is a small shrub that does not reach more than 60 cm and is also commonly used for ornamental purposes [13,18]. Its content of tannins, flavonoids, and phenols has been described to some extent, along with its cytotoxic and antioxidant activities [20]. Another endemic species of North America but less described is *Cuphea cyanea*. It is popular for ornamental purposes as a vine with flowers resembling Christmas lights, from which it got its colloquial name “Serie de Luz (light series)”. Nevertheless, the description of its secondary metabolism is limited. Likewise, the literature on the use of aquaponic systems to grow herbaceous medicinal plants, including identifying, quantifying, or characterising their phenolic content and antioxidant activity is scarce. According to this evidence, this article aimed to analyse the effect of an aquaponic system integrated with Koi carp (*Cyprinus carpio*) and its effect on the content of the bioactive compound such as phenols flavonoids apigenin content, and antioxidant activity of these two medicinal plants “False Heather” (*Cuphea hyssopifolia*), and “Light series" (*Cuphea cyanea*).

Results

Integrated Agri-Aquaculture System performance

Per guidelines described by Palm et al. (2018), due to its size, the system enters the first category of aquaponics (≤ 50 m^2), and according to its design, it can be used domestically, recreationally, or in a backyard. For this domestic vertical aquaponic system, the leakage and flow, drainage, and sedimentation tests of solids without the aquatic organism lasted 15 days. No significant changes or glue were needed to fix the lids on the tubes for NFT. In the following 15 days, the aquatic organism was introduced, and tests related to the accumulation of food were made. The flow within the NFT tubes (56 cm^3 s^{-1}) and the return of water to the fish tank resulted in oxygen levels above 7 mg L^{-1}, an adequate level for the carp. Similarly, the 1-inch hoses to recirculate water showed no blockages due to solids and sediment. The pump had enough power to carry water to the 36 plants on all three levels. No fish mortality occurred during the study.

- Water quality in the coupled aquaponic system

Water temperature, pH, DO, and EC concentrations varied between 17–32 ºC, 8.6–9.3, 6- 8.5 mg L^{-1}, and 225–280 µS $cm^{-1,}$ respectively. The average water hardness values in the fish tank fluctuated between 50–90 mg L^{-1} of Ca, 10–30 mg L^{-1} of Mg, and 30–35 mg L^{-1} of K; and for Cl, PO_4-P and SO_4-S, the values

were < 0.5 mg L^{-1}. These initial values were similar in both tanks before carps were introduced. However, once the 30-day test with the fish ended and the medicinal plants were added, the water showed an accumulation of certain compounds while others decreased in concentration. In the Coupled Aquaponic System with *C. hyssopifolia* (AqH), the K concentration decreased by almost 80%. At the same time, the Mg was almost three times higher, the SO_4-S was 3.7 times higher, the PO_4-P was four times higher, and the NO_3-N was the highest with 6.5 times more concentration. The Cl, NH_4-N, and NO_2-N remained at < 0.2 mg L^{-1}. At the end of the trial time, the released rate (mg L^{-1}) of nutrients in the water derived from fish feeding was in decreasing order NO_3-N(130) > Mg(90) > SO_4-S(64) > PO_4-P(9) > K(2) > Cl(0.28) > NH_4-N(0.1) > NO_2-N(0.04).

For the Coupled Aquaponic System with *C. cyanea* (AqC), the concentration of K, Mg, Cl, NH_4-N, and NO_2-N had similar values to AqH. At the same time, PO_4-P(1.58 mg L^{-1}) remained constant over time, and the SO_4-S decreased slightly (15 – 11 mg L^{-1}), whereas NO_3-N did not behave the same, dropping by 70%. At the end of the trial time, the released rate (mg L^{-1}) of nutrients for AqC was Mg(80) > NO_3-N(6) > SO_4-S(11) > PO_4-P(1.50) ≈ K(1.58) > Cl(0.23) > NH_4-N(0.1) > NO_2-N(0.008).

- Growth and development of Cuphea spp.

There were no significant differences in the growth of the species in the aquaponic and conventional systems. Regarding its percentage of humidity in the greenhouse, the samples from AqH showed 59.67% humidity at the beginning of the test while once the experiment was finished it had 43.26%. The AqC samples had 81.31% humidity while at the end of the test period they only had 51.92%. According to the geographical zone descriptions where *Cuphea* growth the temperature (29.02 ± 9.48 ºC) and humidity (53 ± 8.05%) within the protected system were at their tolerable limits [13]. Table 1shows the results observed for the length of their branches and their leaf area.

Table 1. Growth and development of *Cuphea* spp. Measures at the time zero an after 30 days of trial in coupled aquaponic system. Data are means ± SD for 5 replicates for each system in each level of NFT tubes.

Plant species		Maximum branch height	Leaf area
C. hyssopifolia	Time Zero	29.71 ±8.1[a]	1.42±0.7[a]
	Aquaponic	39.98±10.3[a]	1.86±0.5[a]
	Conventional	30.05±5.2[a]	1.87±0.2[a]
C. cyanea	Time Zero	77.76±40.1[a]	19.16±1.4[a]
	Aquaponic	69.78±32.9[a]	22.07±3.8[a]
	Conventional	65.04±19.7[a]	23.74±8.5[a]

1. Bioactive compounds

The cultivation method generated differences in the concentration of bioactive compounds (Fig. 1) and the antioxidant activity of the evaluated *Cuphea* species (Fig. 2). For *C. hyssopifolia* in its acclimatization stage (Time zero-T0) it was shown to have the highest concentration in both non-volatile second metabolites, 80.39 ± 9.9 mg GAEq and 10.71 ± 1.0 mg CAEq of phenolic and flavonoid respectively. Approximately 76% of phenols and more than half of the flavonoids remained in the dry basis of the plant cultivated in aquaponic systems. While the content of the compounds when remaining in conventional cultivation was reduced by 80%. In the case of *C. cyanea*, the phenolic content at acclimatization time was 17.06 ± 0.8 mg GAEq over time remained above 80% in both types of crops; and its flavonoic content in aquaponic system decreased by 53%.

Elgindi et al., (2011) described that 35 flavonoids were found in 16 *Cuphea* spp, however the presence of catechin, and caffeic acid, and p-coumaric acid has not been described. So, they were proposed in this study in case they could be detected through UPC^2 method. Although it has written that *C. hyssopifolia* has been isolated kaempferol, catechin, quercetin, is this study we do not find any of the above, but well we find apigenin (Fig. 3) which has only been described in a limited number of *Cuphea* spp. [18]. The results show significant differences between treatments. At the beginning of the experiment the concentration of apigenin in *C. hyssopifolia* was 1.06 mg g^{-1} whilst its content at the end of the test compared increased more than 60% (1.63 mg g^{-1}), while its concentration in conventional cultivation decreased by about a 93% (0.10 mg g^{-1}). Regarding *C cyanea*, its apigenin concentration started with 2.2 mg g^{-1}, a higher concentration than *C hyssopifolia*, Although, at the trial end time decreased approx. 40% (0.89 mg g^{-1}). In its concentration on conventional culture decreased 97% (0.0067 mg g^{-1}). It should be noted that for both species their values in apigenin concentrations were closer in the beginning of the experiment, not being so in their content of phenols and total flavonoids. However, at the end of the test time in conventional cultivation in both species the apigenin content decreased almost entirely but remaining at a considerable percentage in AqH.

Figure 2. Antioxidant activity of (**A**) *Cuphea hyssopifolia* and (**B**)*Cuphea cyanea* at Time zero (T0) and at final trial in Aquaponic and Conventional culture. Data are means ± SD for 3 replicates for each system.

Discussion

It is important to keep in mind that the morphology of the two species is different even though they belong to the same genus. The content of water in *C. hyssopifolia* change around 16% since the time zero to the end, and *C. cyanea* decreased in its water content near 30% from time zero to last day. According to Graham (1994) *C. hyssopifolia* has a root system of a short primary root and many lateral of equal thickness, and the tertiary root is fibrous while *C. cyanea* is not fully described of its root system, which we observed, is less fibrous and abundant. *C. cyanea* needed manipulation during the first week of the experiment because its long and creeping leaves moved the roots out of the NFT tube, after this period and together with the sediment that accumulated in the roots it could be kept in place. At the end of the experiment, *C. cyane*a showed death of flowers, then of leaves, and at the end, of complete branches,

although not so of the complete plant. Some of the same problems were also reported by Abdel-Rahim (2019) where of the four medicinal plants that were produced, only mint and rosemary survived until the end of the 30-day period. They showed a rot of the roots in thyme and marjoram like a gel due to the dissolution of the diet and sedimentation of fish feces that ended up covering the roots. In this study, we observed that effect only in certain parts of the roots of *C. hyssopifolia*, whereas the other roots looked healthy, not so in *C cyanea* that had more parts with this gel. This is probably because the method we used was NFT and not floating bedding, which allowed the roots to have new shoots into the air. At the end of the trial test, only *C. hyssopifolia* showed "full bloom" (floral and leaf growth), as described by Berti et al. (2008). This is a confirmation for later uses in aquaponic systems because, together with Charoenkit and Yiemwattana (2017) findings, *C. hyssopifolia* presents good characteristics to be used as living walls reducing the temperature by an average of 2.4 ºC and collaborating on carbon sequestration. While for the use of *C. cyanea* the design within aquaponic systems should be reconsidered either in its use with inert substrate or with another aquaculture species.

Due to limitations in studies of this native species on growth in different media, methods, and nutrient solutions, it is not possible to directly compare the effect of aquaponic systems on *Cuphea* spp. Yang et al. (2019) report the effect of aquaponic systems on basil growth, but the overall growth of basil is different from these species. The growth of *Cuphea* has about one year of life with continuous flowering, so its comparison is not very useful with other medicinal plants or shrubs, not to mention that most of the experimental tests are carried out in systems with larger densities and mostly with tilapia.

The cultivation method produces differences in the concentration of the phytochemical profile of *Cuphea* spp [24]. This is relevant since, in different studies, a content of compounds is reported for a species of *Cuphea* and then generalised for the whole genus [14,22,25]. Santos, Henriques and Mendez (2021) review the relevant literature about *Cuphea* spp. but there is only information of this type for 12 species of the more than 260 described, not including *C cyanea*. Therefore, this study provides the first approach to the compounds in *C. cyanea.* Furthermore, our most outstanding finding is that the levels of polyphenols change significantly in both species due to their integration into the aquaponic systems. Other studies on *Cuphea* spp. focus mainly on its open cultivation and were conducted on a few commercially important species such as *C. hookeriana* and *C. painteri*; *C. ignea* and *C. llavea*, and *C. carthagenensis*[26,27]. However, the species studied here are mainly medicinal and recognised by local herbalists to cure ailments, as an insecticide, and treat sore throats [18]. These species also possess specific activities, such as an antitumor effect on human promyelocytic leukemia (HL-60 cells) [28] and a decrease in the effect on lipid peroxidation due to paracetamol-induced hepatoxicity [29].

In their study, Flanigan and Niemeyer (2014) describe that the variety affects the composition of the bioactive compounds and Oladimeji et al. (2020) report that using an inert substrate as a culture medium modifies water quality and, therefore, nutrient accumulation and secondary metabolite content. While this aspect needs further investigation, it suggests that *C. cyanea* can grow in aquaponic culture if some variables are modified, such as the aquatic species, the use of some inert substrate as a support medium

for the roots, water temperature, etc. It is plausible that the decreased growth and death of *C. cyanea* occurred due to a distressing effect. Polyphenols in plants and their antioxidant activity are beneficial for human health. Thus, an increase in their content is beneficial. Nevertheless, in plants, these compounds are a part of the defensive response to stress [32]. Therefore, an unusually high amount of polyphenols, which occurs in an attempt of controlling oxidative damage, may be associated with a decrease in plant growth [33]. In this case, the distressing effect might be related to nutrient availability. According to Olness et al. (2005), when this genus is grown hydroponically, deficiency in root growth is manifested due to a lack of nutrients (e.g., vanadium) or ionic ratios. In this study, *C. cyanea* showed the same result of low root growing, although further studies on the nutrient dynamics in aquaponics with medicinal plants are needed to clarify these findings. Basil (*Ocimum basilicum*) presents morphological characteristics like *C. hyssopifolia* (e.g., shrub type, pivoting root, antioxidant properties correlated with disease prevention in humans), and parsley (*Petroselinum crispum*), on the other hand, is identified as a condiment or herb with beneficial functions in health due to its content of phenolic acids and flavonoids [35]. In the study conducted by Braglia et al., (2022) with ornamental fish and both the last-mentioned species, they found that the growing method has a significant effect on plant performance. Concerning the flavonoid content in parsley, aquaponics caused a significant increase in quercetin. Also, a remarkable increase was reported in other compounds such as myricetin and rosmarinic acid (+ 1861% and 633%, respectively).

Biostimulants are compounds of biotic origin that can induce a pre-stress conditioning effect, which promotes various physiological responses. The stimulating response in a generated way reaches values between 30–60% above the values that are reported for the control [36]. In this study we found the levels of apigenin increase more than 60% in AqH, so we can confirm eustress effect related to cultivation by IAAS and its consequent increase in the production of bioactive compounds. In addition, during the analysis of the samples by the UPC^2 method, peaks for different flavonoids were detected without identification because the standards were not available (data not shown). Apigenin is a natural flavonoid found in medicinal plants and other fruits and vegetables. It is recognised for being found in large quantities in garlic, chamomile, orange, and propolis [37]. Its importance lies in its biological functions beneficial to human health, i.e., antitumor effect, beneficial for the cardiovascular system, and the effect on the liver, respiratory, endocrine, and central nervous systems. Apigenin acts specifically as an anti-inflammatory, antibacterial, antiviral, antiallergic, cytotoxic, antitumor, and as a treatment of neurodegenerative diseases [38]. This study shows that *C. hyssopifolia*, in AqH, has appropriate synergy and that cultivation under aquaponic conditions has biostimulant effects maintaining the shikimate and the acetate pathways on [39]. This allowed phenotypically better development than *C. cyanea* in the AqC.

The result obtained in the present study showed that the aquaponic system design is suitable for keeping *Cuphea* spp. in a greenhouse or indoor conditions. AqH system (*Cyprinus carpio- Cuphea hyssopifolia*) was better for maintaining growth, high conversion of ammonia to nitrates in the water and polyphenolic compound concentration in the plants, as well as increasing a specific flavonoid, apigenin, compared with the conventional culture. It indicates that aquaponic cultivation can promote the biostimulation of medicinal plants. More studies regarding the elicitor and biostimulant effect of aquaculture wastewater

and its response in plants are necessary to confirm the type of stress, eustress or distress, is caused by the use of aquaponics [40]. In this case, the aquaponic integration of *Cuphea* spp. with *Cyprinus carpio* increases the production of polyphenolic compounds, each variety in a specific concentration. It was observed that *Cuphea cyanea* is not an excellent candidate to be introduced into aquaponic systems, at least not without an inert substrate or other media in NFT. Considering the limited data concerning medicinal plants, including *Cuphea* spp., in different growing methods, the findings reported here contribute toward the use of aquaponics as a sustainable system for producing higher quality food.

Materials And Methods

Integrated Agri-aquaculture systems

For the Integrated Agri-aquaculture systems design, a vertical aquaponic system with Nutrient Film Technique (NFT) was considered (Fig. 4). A metallic structure (2 m x 1 m) was used to support the system, which consisted of three plastic tubes with 12 plants per level, for a total of 36 plants per treatment. Two submersible pumps (30 W; 1 Hp; 56 L min^{-1}) were used, and they were left on from the beginning until the end of the experiment (60 days). Two fish tanks (40 cm x 40 cm x 100 cm) were filled to 160 L each. Oxygenation was carried out by returning the water to the tank by gravity. The flow within the NFT tubes of the system was 56 cm^3 s^{-1}. The three tubes together returned a total volume of 1.73 cm^3 s^{-1}, which is in the range of tolerance [31,32]. A 6% daily replacement with fresh water was made, according to Kloas et al. (2015).

- Experiment Location

The experiment was carried out in the Faculty of Engineering, Campus Amazcala of the Universidad Autónoma de Querétaro (20° 31'- 20° 58' N 100° 09'-100° 24' W, 1,850 m a.s.l.). The system was installed in a 504 m^2 multi-tunnel type greenhouse, with a galvanised metal structure covered with polyethylene plastic, 30% of shadow and white anti-aphid mesh windows. The ventilation was passive and without heating. An extra 50% shadow mesh was used to reduce radiation. Table 2A shows the environmental conditions for the 90 days of the experiment (February to April), of which 30 days were intended for the construction and hydraulic tests of the system, 30 days for the acclimatisation of the carp and the last 30 days for testing the integration of organisms in the system.

Table 2. A) Microclimate conditions; B) Water conditions *in situ* of a greenhouse at the Amazcala Experimental Campus. *The value was obtained from outside of the greenhouse.

A)

Variable	Data
Temperature greenhouse (°C)	29.02 ± 9.48
Radiation* (W/m^2)	163.6 ± 42.06
Relative Humidity (%)	53 ± 8.05

B)

Variable	Data
Temperature fish tank (°C)	24.62 ± 5.04
pH	8.95 ± 0.20
Oxygen (mg L^{-1})	7.23 ± 0.88
Conductivity (µS cm^{-1})	248.52 ± 14.22

- Water quality

The Electric Conductivity (EC), pH, and Dissolved Oxygen (DO) were monitored twice a week. The DO of the water in the systems was determined with a multiparametric meter HQ40D (HACH, USA) with the sensor LDO101-03 (°C and DO) and EC (series-H, °C, and µS cm^{-1}). The pH was measured with the waterproof pH tester 10 sensor (EUTECH, USA Instruments). An initial analysis of the water quality (ammonium, nitrate, nitrite, phosphorus, and potassium) was carried out with a DR/6000 spectrophotometer (HACH®) using the Hach 380 N method (Table 2B). The baseline (minimum and maximum) fish water hardness before starting the trials (30 days of recirculation) is described in Table 3.

Table 3
Nutrients in the water of 30 days-trial recirculation (hydraulic test) in the coupled aquaponic system in a greenhouse at the Amazcala Experimental Campus; baseline fish water quality before the integration of Koi carp- *Cuphea* spp. *Concentrations in the aquatic phase.

Water Quality (mg L^{-1})*	Min	Max
NH_4-N	0.15	1
NO_3-N	5	22
NO_2-N	0.04	0.045
PO_4-P	0.5	3
SO_4-S	1.5	24
Ca	10	90.5
Mg	11	41
Cl	0.02	0.024
K	26	28

- Aquatic species

An ornamental species in juvenile stage, *Cyprinus carpio* L. var. Koi was obtained from a local provider, “Aquario Biotopic and Garden”. Commercial food (TetraO Poind Koi Vibrance®) was used with crude protein 31.0%, crude fat 5.0%, crude fibre 2.0%, moisture 7.0%, phosphorous 0.9%, and ascorbic acid (Vit. C) 100 mg kg^{-1}. The koi carps were fed at a daily rate of 4% of the total biomass of each tank (78 g) divided into two servings per day described by [7].

- Plant species

Obtaining and Authentication

The plants of *C. hyssopifolia* and *C. cyanea* (Fig. 5A & **B**) were obtained from the greenhouse "Red Viverista" in Cuernavaca Morelos, Mexico (19°00'21.3" N 99°14'57.8" W), from the same batch. MSc Yolanda Pantoja carried out the authentication of the species endorsed by Dr Luis Hernandez-Sandoval, herbal curator, in the *QMEX herbarium* of the Faculty of Natural Sciences of the Universidad Autonoma de Queretaro. These species are not listed under NOM-059-SEMARNAT-2010 as threatened or subject to special protection. The authentication code for *Cuphea hyssopifolia* Kunt was 00006843. The authentication code for *Cuphea cyanea* Moc. & Sessé ex DC was 00006847 (see additional information).

The collection of plant material and the performance of experimental research on such plants complied with the national guidelines of México in the standards: NOM-003-STPS-1999 and NOM-007-STPS-2000.

Samples and treatment of Cuphea **spp.**

- Pre-treatment of samples and monitoring

Sampling was conducted on the 2nd of February 2016, maintaining the original proportion of the plant, and leaf, flower and dry stem were collected. Samples were collected randomly from the apex, in the middle, and at the end of each species. Once the samples were collected, they were weighed and placed in paper bags in an oven at 35ºC for four days. After grinding on a sieve with a 20 mm mesh opening, 500 mg was taken from here for extraction, and the rest was stored in amber plastic bottles at room temperature without exposure to light.

Dry samples (500 mg) were added to 5 mL of a solvent mixture containing 80% methanol, 18% distilled water, and 2% formic acid. After 30 s of vortex agitation, the extracts were sonicated for 30 minutes at room temperature and centrifuged at 8,500 rpm for 15 min at 4°C. The supernatant was recovered, and 5 ml of the solvent mixture was added to the remaining pellet. It was stirred for 30 s in a vortex, sonicated for 30 min at room temperature and centrifuged at 8500 rpm for 15 min. The supernatant was recovered with the above. Once together, the final volume of the extracts was measured, filtered in acrodisc then used in all determinations.

The maximum plant height was recorded for Time Zero (T0) on the 4th of February, a second measurement was made on the 5th of April in the integration with the carp and the last one was recorded at the end of the trial time. The leaf area was monitored and recorded on the same days as follows: for *C. hyssopifolia* 5 leaves from 5 branches were measured randomly from the apex, in the middle, and at the end; for *C. cyanea* 15 leaves from 2 branches were measured randomly from the apex, in the middle and at the end.

- Determination of Total Phenolic compounds

Total phenols were determined by the Folin-Ciocalteu colourimetry method [42] using gallic acid as standard and a 10-point calibration curve. In 2 mL tubes, 100 µL of the extract was added, 400 µL of the solvent (80% methanol + 20% distilled water) with 250 µL of Folin-Ciocalteu reagent (1 N), and after 5 min, 1.25 mL of Na_2CO_3 was added to neutralise. The samples were incubated for 2 h without stirring out of the reach of light and then measured. Absorbance was measured at 765 nm using a Spectra Max reader (Molecular Devices Co., Sunnyvale, USA). Concentrations are given in equivalent milligrams of gallic acid (GAEq) per mL of extract. All assays were performed in triplicate in 2 mL Eppendorf® tubes.

- Determination of Total Flavonoids

Total flavonoids were determined according to the method proposed by Brand-Williams et al., (1995) with (+)-catechin as standard and a 7-point calibration curve. A volume of 300 µL of the standard/extract +

120 µL of distilled water + 90 µL of a 5% $NaNO_2$ solution, and, after 5 min, 90 µL of 10% $AlCl_3.6H_2O$ was added and let stand for 6 min. Afterwards, 600 µL of NaOH (1 M) was added, and the volume was taken to 2.5 mL with distilled water. The solution was mixed, and the absorbance was measured at 510 nm in a Spectra Max reader (Molecular Devices Co., Sunnyvale, USA). Concentrations are given in equivalent milligrams of (+)-catechin (CAEq) per mL of extract.

- Determination of Antioxidant Activity,1-Diphenyl-2-picrylhydrazyl Radical (DPPH) Inhibition Assay.

The determination of the antioxidant activity by the DPPH method [44] was prepared in DPPH (1,1-diphenyl-2-picrylhidrazil) reagent with methanol. Aliquots of 1.865 mL of the reagent were placed in 2 mL microtubes along with 0.135 mL of the methanolic extract of each sample. The mix was allowed to stand for 30 min, protected from light. Trolox was used for the calibration curve, and the reading was performed at a wavelength of 480 nm.

- Determination of Antioxidant Activity ferric reducing/antioxidant power (FRAP) Assay.

To determine the antioxidant activity by the FRAP method [45], the reagent was prepared with a mixture of a 20 mM solution of iron trichloride ($FeCl_3$), acetate buffer with anhydrous sodium acetate, and sodium acetate trihydrate at a pH of 3.7. Finally, TPTZ (tripiridil-2-thiazide) was prepared at 10 mM dissolved in 40 mM hydrochloric acid. A mix of 1.865 mL of the FRAP reagent and 0.135 mL of the methanolic extract of the samples were placed in 2 mL microtubes and allowed to react for 30 min, protected from light. Trolox was used for the calibration curve. The absorbance was read at 630 nm.

- Ultra-Performance Convergence Chromatography, UPC^2

Extraction for identification and quantification

For the analysis of phenolic compounds, 200 mg of dry and finely ground samples were weighed and then 1 mL of methanol (HPLC grade) was added to each and stirred in a Vortex for 30 seconds. Subsequently, the samples were placed in an ultrasonic chamber for 30 minutes at RT and protected from light. After this time, the samples were centrifuged at 9500 rpm for 5 minutes. The supernatant was recovered, and the solid residue was subjected to the same extraction procedure four consecutive times. Finally, the supernatants were pooled, and the total volume was taken to 5 mL. The extract obtained was filtered with an acrodisc and stored in amber vials at -20°C until analysis.

Analysis of phenolic compounds

The analysis of phenolic compounds was carried out by convergence chromatography (UPC^2: Ultra-performance convergence chromatography). A Waters System HPLC chromatograph (Waters Corporation, Milford, MA, USA) was used, which consists of a quaternary pump, a diode array detector (model 996), an online vacuum degasser (MetaChem Technologies Inc.) and a Rheodyne injector (4793). The control of

the equipment, the process, and the management of the chromatographic information was carried out with the Millennium program (Waters). The previously prepared samples were injected into the UPC^2 according to the analysis conditions to determine their chromatographic profile (Table 4). Subsequently, the standards (SIGMA, purity ≥ 95%) apigenin, kaempferol, catechin, quercetin, caffeic acid, and p-coumaric acid were injected to decide its retention time and obtain their UV spectra. The retention times and UV spectra of the different peaks in the samples were compared with those of the standards. Coincident peaks were subjected to co-elution to confirm the correspondence of the compounds.

Table 4
Method for detecting the phenolic compounds of *Cuphea* spp. with the UPC^2. Conditions in which the samples were introduced: Injection volume: 10 µL, Flow rate: 1.5 mL min^{-1}, Column: Viridis BEH 5µm, 4.6 X 100 mm, Column temperature: 40°C, ABPR: 1500 psi. *CO_2 Coleman grade.

Time (Min)	*CO_2 (%)	Methanol (%)
0	95	5
8	70	30
9	70	30
10	95	5
11	95	5

Statistical Analysis

Three biological replicates were processed for the measurements of bioactive compounds, and three technical replicates were performed for each assay. The results are reported as the mean ± standard deviation (SD). A one-way ANOVA and a Tukey means comparison test ($p \leq 0.05$) was performed using the Statgraphics Centurion 19 software.

Declarations

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Author contributions statement

P FA; Writing—original draft preparation, Investigation; A RC, Formal analysis, Writing—editing; E RdL. Resources, data curation, editing; M TA, Resources, visualization and validation; G SZ, Supervising, Project administration, and methodology.

Competing interest

All authors reviewed the manuscript and report there are no competing interests to declare.

Additional information

All data generated and analysed during this study are included in this published article in the file *supplementary_material_cuphea2023.*

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References

1. Bartelme, R. P., Oyserman, B. O., Blom, J. E., Sepulveda-Villet, O. J. & Newton, R. J. Stripping away the soil: Plant growth promoting microbiology opportunities in aquaponics. *Front Microbiol* **9**, 8 (2018).
2. Delaide, B., Teerlinck, S., Decombel, A. & Bleyaert, P. Effect of wastewater from a pikeperch (Sander lucioperca L.) recirculated aquaculture system on hydroponic tomato production and quality. *Agric Water Manag* **226**, (2019).
3. Braglia, R. *et al.* Phytochemicals and quality level of food plants grown in an aquaponics system. *J Sci Food Agric* **102**, 844–850 (2022).
4. Suhl, J. *et al.* Advanced aquaponics: Evaluation of intensive tomato production in aquaponics vs. conventional hydroponics. *Agric Water Manag* **178**, 335–344 (2016).
5. Palm, H. *et al.* Towards commercial aquaponics: a review of systems, designs, scales and nomenclature. *Aquaculture International* vol. 26 813–842 Preprint at https://doi.org/10.1007/s10499-018-0249-z (2018).
6. Gooley, G. J., Gavine, F. M. & Rural Industries Research and Development Corporation (Australia). *Integrated agri-aquaculture systems : a resource handbook for Australian industry development*. (Rural Industries Research and Development Corp, 2003).
7. Rakocy, J., Masser, M. & Losordo, T. *Recirculating Aquaculture Tank Production Systems: Aquaponics-Integrating Fish and Plant Culture*. http://osufacts.okstate.edu (2016).
8. Forchino, A. A., Lourguioui, H., Brigolin, D. & Pastres, R. Aquaponics and sustainability: The comparison of two different aquaponic techniques using the Life Cycle Assessment (LCA). *Aquac*

Eng **77**, 80–88 (2017).

9. Verdoodt, S. *Life cycle assessment of aquaponics: A case study in the urban environment*. (2018).
10. Yep, B. & Zheng, Y. Aquaponic trends and challenges – A review. *Journal of Cleaner Production* vol. 228 1586–1599 Preprint at https://doi.org/10.1016/j.jclepro.2019.04.290 (2019).
11. Zarantoniello, M. *et al.* Physiological responses of Siberian sturgeon (Acipenser baerii) juveniles fed on full-fat insect-based diet in an aquaponic system. *Scientific Reports 2021 11:1* **11**, 1–13 (2021).
12. Yang, T. & Kim, H. Characterizing nutrient composition and concentration in tomato-, basil-, and lettuce-based aquaponic and hydroponic systems. *Water (Switzerland)* **12**, (2020).
13. Graham, S. A. & Sosa, V. Flora de Veracruz.". *Lytraceae". Fascículo* **66**, 15–24 (1991).
14. Santos, M. C., Henriques, A. T. & Mendez, A. S. L. Analytical methods of phytochemicals from the Cuphea genus - A review. *Drug Analytical Research* **5**, 4–10 (2021).
15. Graham, S. A. & Kleiman, R. Composition of seed oils in some Latin American Cuphea (Lythraceae). *Ind Crops Prod* **1**, 31–34 (1992).
16. Graham, S. A., Hirsinger, F. & Röbbelen, G. Fatty acids of Cuphea (Lythraceae) seed lipids and their systematic significance. *Am J Bot* **68**, 908–917 (1981).
17. Gesch, R., Forcella, F., Olness, A., Archer, D. & Hebard, A. Agricultural management of cuphea and potential for commercial production in the Northern Corn Belt. *Ind Crops Prod* **24**, 300–306 (2006).
18. Elgindi, M., Ayoub, N., Milad, R. & Hassan, R. A comprehensive review of Cuphea (Lythraceae). *Res J Pharm Biol Chem Sci* **2**, 847–856 (2011).
19. Tiwari, D. K., Coria-Tellez, A. V., Tripathi, D., Alonso-Perez, M. O. & Navarro-Pérez, A. D. Extraction and Characterization of chemical constituents present in Cuphea aequipetala and their properties. *Microscopy and Microanalysis* **27**, 2278–2282 (2021).
20. Tungmunnithum, D., Thongboonyou, A., Pholboon, A. & Yangsabai, A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines* **5**, 93 (2018).
21. Abdel-Rahim, M. M., Awad, Y. M., Abdallah, Y. A. & Radwan, S. M. *Effects of four medicinal plants on the bioeconomic analysis and water-use efficiency of Nile tilapia, Oreochromis niloticus fry nursed under a small-scale aquaponics system*. vol. 12 http://www.bioflux.com.ro/aacl (2019).
22. Berti, M. T. & Johnson, B. L. Growth and development of cuphea. *Ind Crops Prod* **27**, 265–271 (2008).
23. Yang, T. & Kim, H. J. Nutrient management regime affects water quality, crop growth, and nitrogen use efficiency of aquaponic systems. *Sci Hortic* **256**, 108619 (2019).
24. Khandaker, M. & Kotzen, B. The potential for combining living wall and vertical farming systems with aquaponics with special emphasis on substrates. *Aquac Res* **49**, 1454–1468 (2018).
25. Sharratt, B. S. & Gesch, R. W. *Rooting Characteristics and Water Requirements of Cuphea*. (2002).
26. Graham, S. A. & Knapp, S. J. Cuphea: A new plant source of medium-chain fatty acids. *http://dx.doi.org/10.1080/10408398909527495* **28**, 139–173 (2009).

27. Bertram, R. I. *et al.* Search for new industrial oils, IX.Cuphea, a versatile source of fatty acids. *Journal of the American Oil Chemists' Society 1964 41:4* **41**, 279–280 (1964).
28. Elgindi, M., Ayoub, N., Milad, R. & Mekky, R. Antioxidant and Cytotoxic Activities of Cuphea hyssopifolia Kunth (Lythraceae) Cultivated in Egypt. *J Pharmacogn Phytochem* **1**, (2012).
29. Moke, E. G., Mordi, J. C. & Umukoro, E. K. Effects of Methanol Leaf Extract of Cuphea Hyssopifolia Kunth on Liver Enzymes Activity and Antioxidant Indices of Paracetamol-Induced Hepatotoxicity in Wistar Rats. *African Journal of Biomedical Research* **23**, 123–126 (2020).
30. Flanigan, P. M. & Niemeyer, E. D. Effect of cultivar on phenolic levels, anthocyanin composition, and antioxidant properties in purple basil (Ocimum basilicum L.). *Food Chem* **164**, 518–526 (2014).
31. Oladimeji, A. S., Olufeagba, S. O., Ayuba, V. O., Sololmon, S. G. & Okomoda, V. T. Effects of different growth media on water quality and plant yield in a catfish-pumpkin aquaponics system. *J King Saud Univ Sci* **32**, 60–66 (2020).
32. Nozzi, V., Graber, A., Schmautz, Z., Mathis, A. & Junge, R. Nutrient management in aquaponics: Comparison of three approaches for cultivating lettuce, mint and mushroom herb. *Agronomy* **8**, (2018).
33. Hichem, H. & Mounir, D. Differential responses of two maize (Zea mays L.) varieties to salt stress: changes on polyphenols composition of foliage and oxidative damages. *Ind Crops Prod* **30**, 144–151 (2009).
34. Olness, A., Gesch, R., Forcella, F., Archer, D. & Rinke, J. Importance of vanadium and nutrient ionic ratios on the development of hydroponically grown cuphea. *Ind Crops Prod* **21**, 165–171 (2005).
35. Ferreira, F. S. *et al.* Bioactive compounds of parsley (Petroselinum crispum), chives (Allium schoenoprasum L) and their mixture (Brazilian cheiro-verde) as promising antioxidant and anti-cholesterol oxidation agents in a food system. *Food Res Int* **151**, (2022).
36. Hernández, M. V. & Jiménez, N. I. F. Introducción a la teoría del estrés e inmunología vegetal. in *Manejo del estrés vegetal como una estrategia para una agricultura sostenible* 53–102 (Editorial Universidad de Almería, 2022).
37. Zhou, X., Wang, F., Zhou, R., Song, X. & Xie, M. Apigenin: A current review on its beneficial biological activities. *J Food Biochem* **41**, e12376 (2017).
38. Ali, F., Rahul, Naz, F., Jyoti, S. & Siddique, Y. H. Health functionality of apigenin: A review. *http://dx.doi.org/10.1080/10942912.2016.1207188* **20**, 1197–1238 (2016).
39. Bravo, L. Polyphenols: Chemistry, Dietary Sources, Metabolism, and Nutritional Significance. *Nutr Rev* **56**, 317–333 (1998).
40. González, R. G. G. & Pacheco, I. T. *Manejo del estrés vegetal como una estrategia para una agricultura sostenible*. vol. 3 (Universidad Almería, 2022).
41. Kloas, W. *et al.* A new concept for aquaponic systems to improve sustainability, increase productivity, and reduce environmental impacts. *Aquac Environ Interact* **7**, 179–192 (2015).

42. Oomah, B. D., Cardador-Martínez, A. & Loarca-Piña, G. Phenolics and antioxidative activities in common beans (Phaseolus vulgaris L). *J Sci Food Agric* **85**, 935–942 (2005).
43. Brand-Williams, W., Cuvelier, M. E. & Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology* **28**, 25–30 (1995).
44. Liu, J. K., Hu, L., Dong, Z. J. & Hu, Q. DPPH Radical Scavenging Activity of Ten Natural p-Terphenyl Derivatives Obtained from Three Edible Mushrooms Indigenous to China. *Chem Biodivers* **1**, 601–605 (2004).
45. Singleton, V. L. & Rossi, J. A. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am J Enol Vitic* **16**, (1965).

Figures

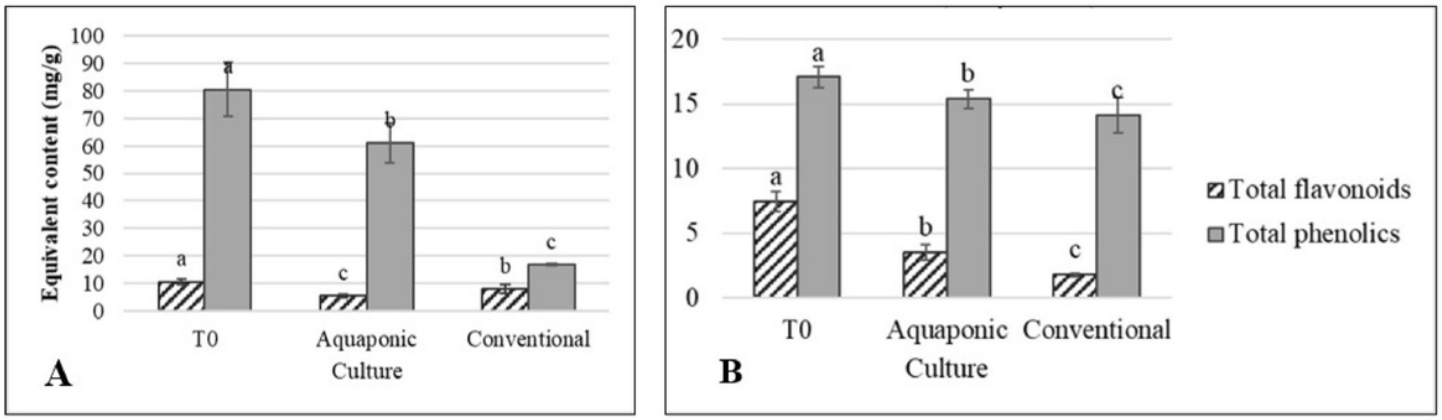

Figure 1

Total phenolic and flavonoid content of (**A**) *Cuphea hyssopifolia* and (**B**) *Cuphea cyanea* at Time zero (T0) and at final trial in Aquaponic and Conventional culture. Data are means ± SD for 3 replicates for each system.

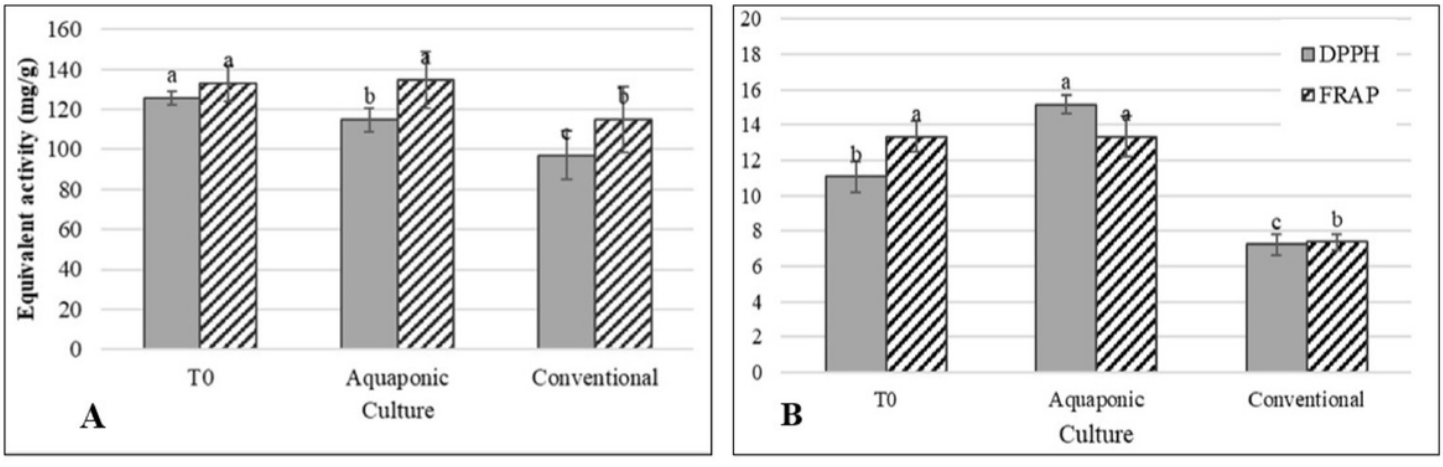

Figure 2

Antioxidant activity of (**A**) *Cuphea hyssopifolia* and (**B**) *Cuphea cyanea* at Time zero (T0) and at final trial in Aquaponic and Conventional culture. Data are means ± SD for 3 replicates for each system.

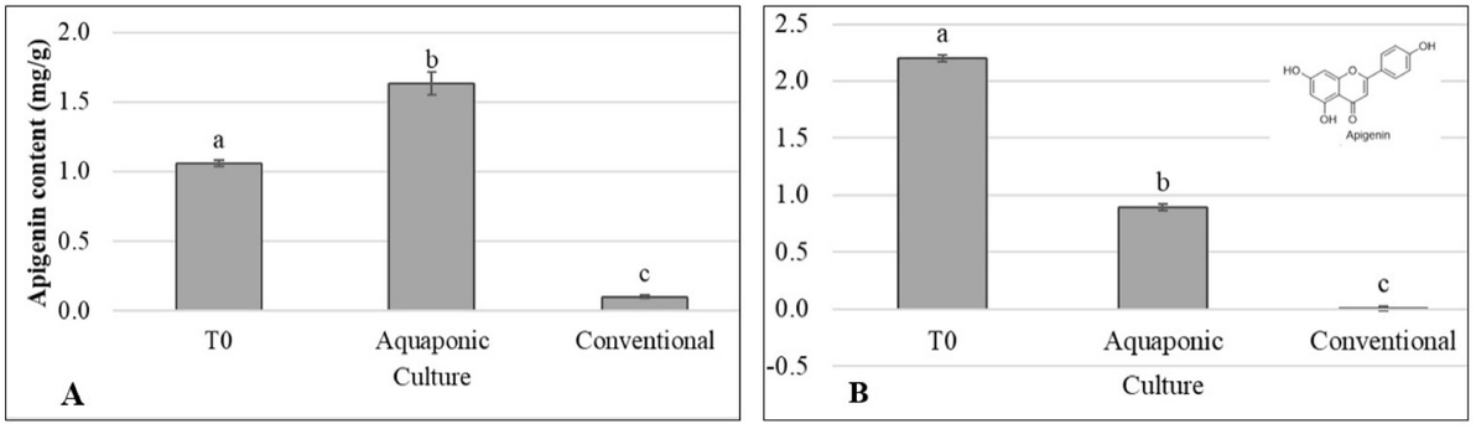

Figure 3

Apigenin content of (**A**) *Cuphea hyssopifolia* and (**B**) *Cuphea cyanea* at Time zero (T0) and at final trial in Aquaponic and Conventional culture. Data are means ± SD for 3 replicates for each system.

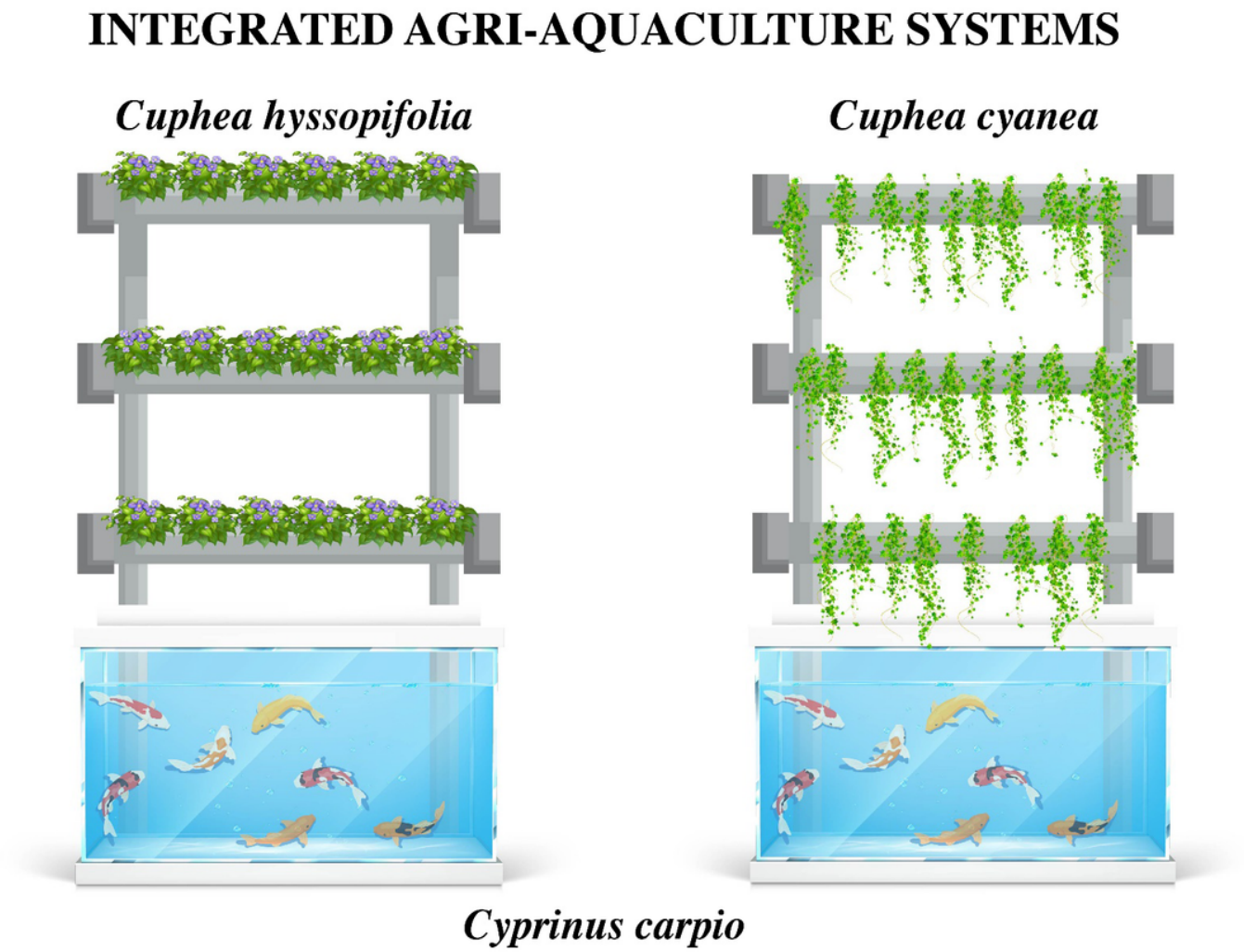

Figure 4

Schematic diagram of the conventional culture (control) and an Integrated Agri-aquaculture System Koi carp-*Cuphea* spp.

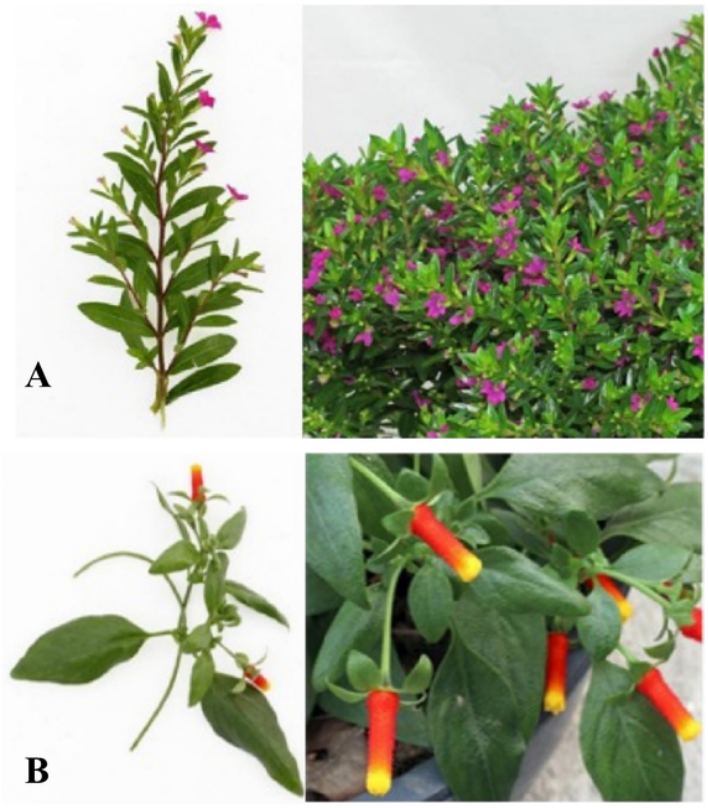

Figure 5. A) *Cuphea hyssopifolia* Kunt—authentication code 00006843.; B) *Cuphea cyanea* Moc. & Sessé ex DC—authentication code 00006847 (see additional information); collection date February 2016, species acquired from "Red viverista" Located in Cuernavaca Morelos, Mexico (19°00'21.3" N 99°14'57.8" W).

Figure 5

See image above for figure legend

Supplementary Files

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